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COMPARATIVE DIVERSITY AND COMPOSITION OF NITROGEN-FIXING CYANOBACTERIA IN THREE DIFFERENT LAND USE SYSTEMS OF UPPER ASSAM

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Abstract

Three contrasting land use systems: reserve forests, rice fields and coal fields located at Upper Assam region of North East India were explored for documenting diversity and species composition of N_2-fixing cyanobacteria. Altogether 24 taxa (16 heterocystous and 8 non-heterocystous) belonging to nine different genera were isolated. The Shannon's diversity index was highest in rice fields (1.946), followed by reserve forest (1.485) and coal fields (0.975). Results of relative abundance revealed the dominance of Nostoc and Anabaena in the reserve forests and rice fields, whereas both were missing in the coal contaminated sites. Oscillatoria was the dominant genus and the species belonging to this genus were abundant in coal field areas. Canonical Correspondence Analysis revealed that environmental factors and physico-chemical properties cumulatively decided the composition and distribution of cyanobacteria in different land use systems of the region.

Key Words: *Diversity, Cyanobacteria, Land use, Reserve forest, Rice fields, Coal fields*

Introduction

Cyanobacteria are Gram-negative photosynthetic autotrophs, widely grown in every conceivable habitat including soil, rocks, water and saline environment (Whitton and Potts, 2000). They are polymorphic, prokaryotic microorganisms which may be single celled, colonial or filamentous and resemble with bacteria in cellular organization and with green plants in oxygenic photosynthesis (Stanier and Cohen Bazire, 1977). Some of the cyanobacterial taxa are capable of fixing the atmospheric nitrogen (Stewart *et al*., 1987), hence their presence in soil is thought to maintain the nitrogen-level in the soil (Venkataraman, 1993). Soil is the most important non-aqueous habitat of N_2-fixing cyanobacteria (Zenova *et al*., 1995).

Although N_2-fixing cyanobacteria are highly adapted to different environmental conditions and considered to be important for the nitrogen input to soil, they are rarely dominant in terrestrial ecosystems (Wartiainen *et al*., 2008) and susceptible to environmental condition (Hunt *et al*., 1979). Their diversity is found to vary with the environment, pH, temperature, availability of nutrients and anthropogenic pressure on the soil (Hoffmann, 1989). Any alteration in the soil characteristics lead to the change in their populations in terms of tolerance, abundance, diversity and dominance in their habitat (Nayak and Prasanna, 2007). Thus, any modification in the soil types, pattern of land use and the extent of soil intensification would affect its quality, thereby altering the diversity of N_2-fixing cyanobacteria, a predominant

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constituent of soil microbiota (Budel, 2002).

North-East India is the abode of many rare and important floral and faunal species which makes it a biological hotspot (Myers *et al*., 2000). In recent times, there have been drastic changes in land use practices in the North Eastern region of India, which are considered to have caused definite deterioration in soil and environmental quality of the region (Singh *et al*., 2014). Such deteriorative changes too noticed in Assam, which is one of the prominent states of NE India.

Although there are several studies on the effect of land uses and soil management practices on soil biota as well as soil quality in different regions of the world (Masto *et al*., 2007; Ayoubi *et al*., 2011; Singh *et al.*, 2014), no information is available regarding soil organisms as a whole and N_2-fixing cyanobacterial diversity in particular in different land use systems of Assam region. Study on N_2-fixing cyanobacterial diversity in different habitats hence is the need of the hour. The comparisons of cyanobacterial diversity in different land intensified areas allow us to estimate their degree of similarity and draw conclusions about their distribution. The present endeavour was therefore aimed to find out the diversity and distribution of N_2-fixing cyanobacteria in the Upper Assam region of NE India which has been highly affected due to recent change in land use practices.

Study Sites

The study was conducted in all easternmost districts collectively known as Upper Assam within the geographical boundary of Assam. Assam is surrounded by the foothills of Eastern Himalayas and is located between 24°44′ N to 27°45′ N Latitude and 89°41′ E to 96°02′ E Longitude, covering 2.4% of the geographical area of the country i.e. 78,438 sq.km. Diverse terrestrial and aquatic ecosystems including extreme ones (hot springs, acidic soils etc.) could be encountered within the state. The fast growing population aided with the modern technology lead to the rapid change in the land use practices from the traditional systems such as forests, cultivated lands, crop fields etc. into modern land deteriorating practices which includes coal mines and crude oil exploration within this geographical region. The region is bounded on the north by the Eastern Himalayas, on the east by the Mishmi Hills, on the south by the Naga-Patkai Hills and on the west by the Mikir Hills and Shillong Plateau. The area experience a typical monsoon climate with average annual rainfall as high as 1800 mm. The valley encloses, within its folds, a large human landscape of agricultural fields, human settlements, forests, historical towns and one of the world's largest stretches of tea gardens.

To perform the study, three different land use systems viz; reserve forests, rice fields and coal fields were considered (Figure 1). The reserve forests in the region were dominated by many tall deciduous trees which include *Dipterocarpus marcocarpus*, *Phoebe goalparensis*, *Shorea robusta*, *Messua ferrea* etc. The region has its climatic and physiographic features favourable for rice cultivation and the crop is grown in a wide range of agro-ecological situations. The rice field ecosystem represents a favorable environment for the growth of cyanobacteria, fulfilling the requirements of light, water, temperature and nutrient availability in an optimal manner. Coalfield represents highly intensed areas as a result of mining activities. Geo-coordinates of different sampling sites (Table 1) were also recorded with GPS (Gramin extres).

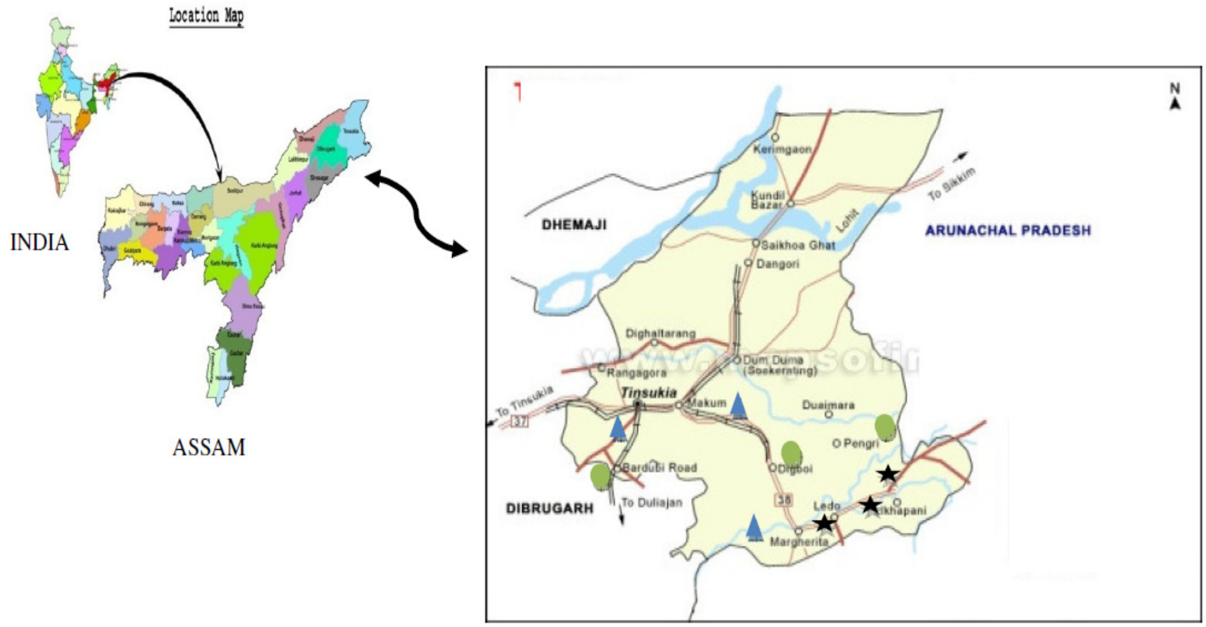

Figure 1: Location of different study areas in and around Upper Assam. Circle represents Reserve forests, triangle represents Rice fields and star represents Coal fields.

Table 1: Geographical location of different study sites

Study Sites	Sampling Sites	Geo-coordinates	Soil Type
Rice Fields	RF 1	26°09’12.7” N 91°39’38.4” E	Loamy Silt
	RF 2	26°16’57.7” N 91°38’33.8” E	Silt
	RF 3	26°16’56.9” N 91°38’30.2” E	Loamy Silt
Reserve Forests	F 1	26°12'12.1" N 91°47'55.2" E	Sandy
	F 2	26°07'11.9" N 91°53'11.4" E	Sandy Loam
	F3	26°13'10.2" N 91°37'31.6" E	Silt
Coal Fields	CF1	26°16'57.7" N 91°38’34.1" E	Sandy
	CF 2	26°16'56.8" N 91°38’30.6" E	Sandy
	CF 3	26°16'56.4" N 91°38’30.6" E	Sandy

Materials and Methods

Isolation, Maintenance and Identification of Cyanobacteria

The soil samples were collected from study sites by clearing the upper litter layer if any. Three samples from the depth of 0–5 cm were taken aseptically at each site at a regular interval of two months. The collected samples were sealed in plastic bags and brought to the laboratory for further studies. The soil samples were air-dried, ground with mortar and pestle and passed through a 2-mm sieve. A pinch of grinded soil were inoculated in freshly prepared nitrogen free BG11 media (Rippka *et al*., 1979) and were allowed to grow under optimal growth condition for 25-30 days at $30^{o} \pm 2^{o}C$ temperatures in 2.3 K lux light intensity at the Ecology Laboratory of Department of Botany, Gauhati university.

Enumeration of cyanobacterial populations was carried out by MPN technique (Pabbi *et al*., 2010). The enrichment flasks and MPN tubes were regularly monitored for growth and the organisms were observed microscopically under a Magnus MLX*i* microscope. Identification was carried out mainly based on morphological characteristics such as cell structure and size, number and position of heterocysts following Desikachary (1959), Anand (1989) and Komarek and Anagnostidis (2005).

Soil Analysis

The soil samples were analysed for different physico-chemical properties. Soil temperature was measured during the time of soil sampling using a soil thermometer. The pH and conductivity of soil were determined with the help of digital pH meter Biochem pm 79 and conductivity meter Systronics 304 respectively following the procedure as outlined by Black (1992). Water holding capacity was estimated following Jackson (1973). Soil characterizations for the amount of available phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), organic carbon and total nitrogen (N) was conducted by following the standard protocols as described by Trivedy and Goel (1987).

Statistical Analysis

Relative abundance

The relative abundance of each cyanobacterial strain was calculated by employing the following formula:

Relative abundance = $\frac{Y}{X} \text{ X } 100$

Where,

X = total number of samples collected

Y= number of samples from which a particular cyanobacteria type was isolated.

Diversity indices

The species diversity was calculated using Shannon- Wiener diversity index, following the formula:

$$H = -\sum_{i=1}^{S} (Pi)\ln(Pi)$$

Where,

H - diversity in a sample of S species or kinds

S - number of species in the sample

Pi - relative abundance of i^{th} species or kinds measures, = ni/N

N - total number of individuals of all kinds

ni - number of individuals of i^{th} species

ln - log to base 2

The similarity analysis of cyanobacteria between the different sites was determined by Jaccard similarity index (J) using the formula: $J= a\ (a + b + c)^{-1}$ (Magurran, 2006), where, *a* represents the number of shared taxa in both samples, *b* is the number of taxa present in one sample, and *c* is the number of taxa in the second sample. The J index would equal to one when all taxa are shared by both the samples and zero when none are shared.

Canonical Correspondence Analysis

The relationship between species distribution and physico-chemical variables of soil was assessed by canonical correspondence analysis (CCA) using the

PAST Software. For this purpose, the generic abundance of the isolated cyanobacterial taxa was taken into account. Soil temperature, pH, conductivity, water holding capacity, P, N, Organic C, Ca, K and Mg were the environmental variables included in the analysis.

Results and Discussion

Physico-chemical Characteristics of Soil

The result of physico chemical analysis of soil samples from different study sites is presented in Table 2. The soil attributes showed differences across the different land use systems. Soil temperature was higher in coalfields (34.26 ± 0.7°C) as compared to that of reserve forests (30.55 ± 1.8°C) and rice fields (29.2 ± 0.5°C). The soil was found to be acidic across all the study sites with the highest pH value recorded at rice fields (6.62 ± 0.08) and lowest at coalfields (4.21 ± 0.14). Conductivity ranged from 65.81 µS ± 2.42 at rice fields to 170.7 µS ± 1.7 at the coalfields. Soil water holding capacity varied from 38.5 ± 2.1 (reserve forests) to 57.3 ± 1.3 (Coal fields). Phosphate content of the soil was also varied in different locations with maximum at rice fields (7.85 ± 0.12 mg/100gm) and minimum at coalfields (3.66 ± 0.67 g/100gm). The organic carbon ranged between 0.68 ± 0.013 percent in reserve forests to 3.0 7 ± 0.007 percent in coalfields. Potassium and nitrogen content were in the range of 10.47±0.26 mg/100gm to 26.15 ± 0.11 mg/100gm and 0.73 ± 0.03 mg/100gm and 2.83 ± 0.16 mg/100gm respectively.

Magnesium content in these sites was comparatively more than calcium content. The mean value of calcium ranged from 34.02 ± 0.22 mg/100gm at coalfields to 71.43 ± 0.25 mg/100gm at rice fields and that of magnesium ranged from 64.51 ± 0.09 mg/100gm (coalfields) to 143.71 ± 0.14 mg/100gm (rice fields).

Table 2: Physico-chemical properties of the soil samples from three different land use systems of Upper Assam

Soil Properties	Reserve Forests	Rice Fields	Coal Fields
Temp (°C)	30.55 ± 1.8	29.2 ± 0.5	34.26 ± 0.7
pH	6.03 ± 0.11	6.62 ± 0.08	4.21 ± 0.14
Cond. (µS)	65.81 ± 2.42	83.46 ± 5.38	170.7 ± 7.13
WHC (%)	40.5 ± 3	38.5 ± 2.1	57.30 ± 1.3
P (mg/100g)	6.65 ± 0.06	7.85 ± 0.12	3.66 ± 0.67
Organic C (%)	0.68 ± 0.013	0.81 ± 0.022	3.07 ± 0.007
K (mg/100g)	19.34 ± 0.17	26.15 ± 0.11	10.47 ± 0.26
Soil N (mg/100gm)	1.76 ± 0.05	2.83 ± 0.16	0.73 ± 0.03
Ca (mg/100g)	62.84 ± 0.32	71.43 ± 0.25	34.02±0.22
Mg (mg/100gm)	112.86 ± 0.24	143.71 ± 0.14	64.51 ± 0.09

N_2-fixing Cyanobacterial Abundance and Diversity

In the present investigation, soil samples were collected from three different land use systems and evaluated for N_2-fixing cyanobacterial abundance and diversity. N_2-fixing cyanobacteria belonging to 9 genera were isolated which included 6 heterocystous forms viz; *Anabaena*, *Nostoc*, *Scytonema*, *Calothrix*, *Rivularia*, *Westiellopsis* and 3 non-heterocystous forms viz; *Lyngbya*, *Phormidium* and *Oscillatoria*. The details of distribution and occurrence of cyanobacterial species identified from each study site are given in Table 3. The members of Nostocales were highly abundant than the members of

Oscillatoriales. Of the total 24 strains isolated, 67% were heterocystous cyanobacteria, of which 25% alone belonging to *Nostoc* and 21% to *Anabaena*.

Comparison of Species Richness and Diversity in Different Locations

N_2-fixing cyanobacterial species richness varied among the different studied locations. Rice fields recorded highest number of species (17), followed by reserve forests (15). Coal fields witnessed lowest species richness and were represented by 6 non-heterocystous species. The abundance of cyanobacteria in different locations could be attributed to favourable contents of available nutrients. Rice fields showed the dominance of the genera *Nostoc* and *Anabaena* with 5 species each, whereas in reserve forest areas, *Nostoc* dominated with 4 species followed by *Anabaena* (3). On the contrary, coalfields showed total absence of heterocystous cyanobacteria and were represented only by the non heterocystous members (Table 3) viz., *Oscillatoria* (3), *Phormidium* (2) and *Lyngbya* (1). *Oscillatoria princeps* was the only species common to each and every site from virgin reserve forests to extremely polluted coal fields. Predominance of *Nostoc* and *Anabaena* in reserve forests and rice fields indicated their competitiveness and ability to occupy diverse habitat. *Oscillatoria* was the most dominant genus in the coalfields as indicated by the abundant value.

Table 3: List of N_2-fixing cyanobacterial taxa isolated from different Land use systems. (+) indicates presence, (-) indicates absence.

S/No.	Cyanobacterial Taxa	Land Use Systems		
		Forests	Rice Fields	Coal Fields
1	*Anabaena variabilis* Kutzing ex Born.et Flah.	+	+	-
2	*A. fertilissima* Rao, C.B.	+	+	-
3	*A. circinalis* Rabenhorst ex Born. et Flah.	+	+	-
4	*A. oryzae* Fritsch	-	+	-
5	*A. doliolum* Bharadwaja	-	+	-
6	*Nostoc linckia* (Roth) Bornet ex Born. et Flah.	+	+	-
7	*N. muscorum* Ag.ex Born.et Flah.	-	+	-
8	*N. punctiforme* (Kutz.) Hariot	+	-	-
9	*N. commune* Vaucher ex Born.et Flah.	+	+	-
10	*N. sphaericum* Vaucher ex Born.et Flah.	+	+	-
11	*N. hatei* Dixit	-	+	-
12	*Lyngbya contorta* Lemm.	+	-	-
13	*L. major* Menegh. Ex Gomont	-	+	+
14	*Phormidium tenue* (Menegh.)Gomont	+	-	+
15	*P. fragile* (Meneghini) Gomont	-	+	+
16	*Oscillatoria princeps* Vaucher ex Gomont	+	+	+
17	*O. willei* Gardner em.Drouet	+	-	-
18	*O. formosa* Bory ex Gomont	-	+	+
19	*O. tenius* C. Agardh ex Gomont	-	-	+
20	*Scytonema rivulare* Borzi ex Born. et Flah.	+	-	-
21	*Calothrix braunii* (A. Br.) Bornet et Flahault	+	+	-
22	*C. marchica* Lemmermann	+	+	-
23	*Rivularia sp.*	+	-	-
24	*Westiellopsis prolifica* Janet	-	+	-

The diversity of N_2-fixing cyanobacteria in different study sites was measured in terms of Shannon-Weiner diversity index. It was observed that cyanobacterial diversity, distribution and species composition in different ecosystems continuously changed with variations in environmental factors and nutrient availability. Among the locations, species diversity of both rice fields and reserve forests was much higher than that of coalfields. Rice fields showed the highest diversity index (1.946) followed by reserve forests (1.485). That was lowest (0.975) in coalfields (Figure 2). Similarly, N_2-fixing cyanobacterial species composition between different sites was calculated using Jaccard's index, which indicated that reserve forests and rice fields were more similar in terms of cyanobacterial diversity. Coalfield on the other hand, was less similar to the other locations (Table 4). Higher values of similarity index indicate the occurrence of more number of common species between two locations. Dissimilarity in Coalfield cyanobacterial communities with the other two sites could be attributed to the difference in soil physico-chemical parameters due to coal generated contaminations and comparatively low range of pH.

Table 4: Similarity index values in different land use systems:

Land Use Systems	Total Isolates	Similarity Index		
		Reserve Forests	Rice Fields	Coal Fields
Reserve Forests	15	-	0.219	0.086
Rice Fields	17	-	-	0.145
Coal Fields	6	-	-	-

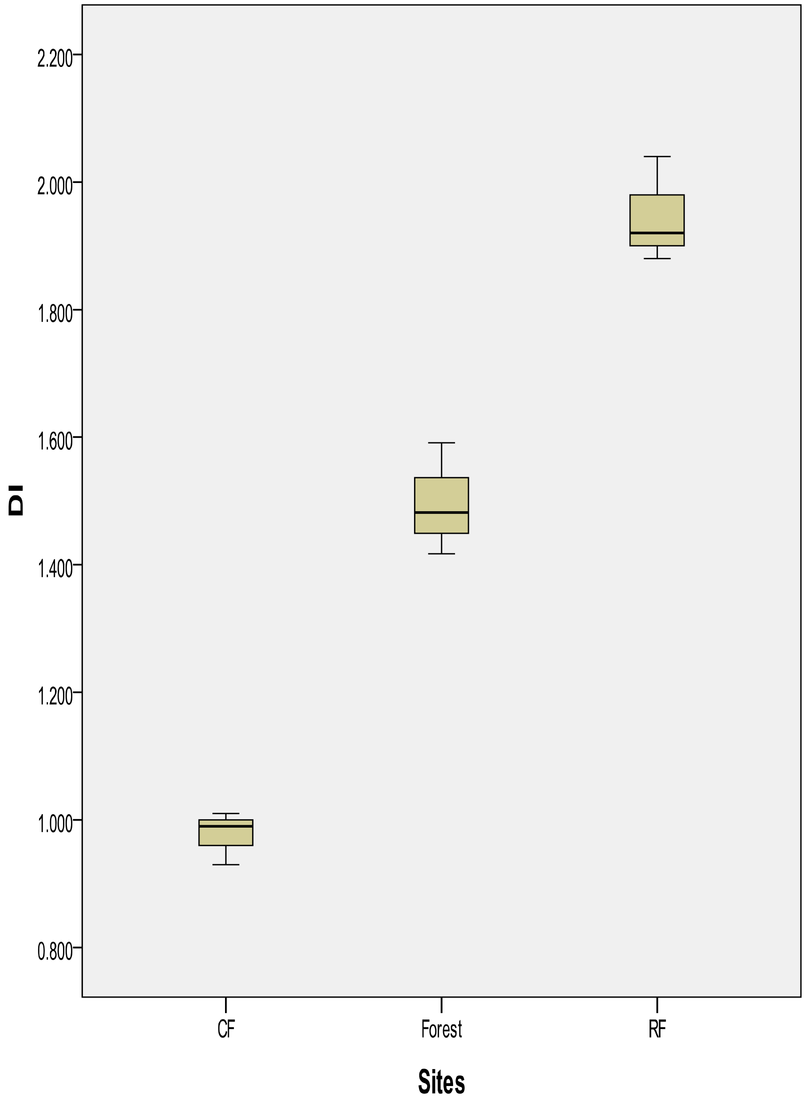

Figure 2: Boxplot of Shannon diversity index (DI) in the three Land use systems- Reserve Forests (Forest), Rice Fields (RF) and Coal Fields (CF) of Upper Assam.

Relationship between Cyanobacterial Abundance and Soil Physico-chemical Parameters

CCA based on N_2-fixing cyanobacterial abundance data and environmental variables was carried out to explore the potential correlation between them. The first two CCA axes had eigen values of 0.21 and 0.08 respectively. The result showed that the distribution of cyanobacteria was associated with different physico-chemical parameters (Figure 3). Species belonging to *Nostoc*, *Anabaena*, *Calothrix*, *Rivularia*, *Scytonema* and *Westiellopsis* were associated with rice fields and reserve forests and showed positive correlation with pH, potassium, soil nitrogen, phosphate and calcium content. *Nostoc* and *Anabaena* were abundant in rice fields and reserve forests where pH, soil N and magnesium content were relatively high. Species of

Oscillatoria, *Phormidium* and *Lyngbya* were positively correlated with coal contaminated sites, which were characterized by high temperature, organic C, soil water holding capacity and conductivity.

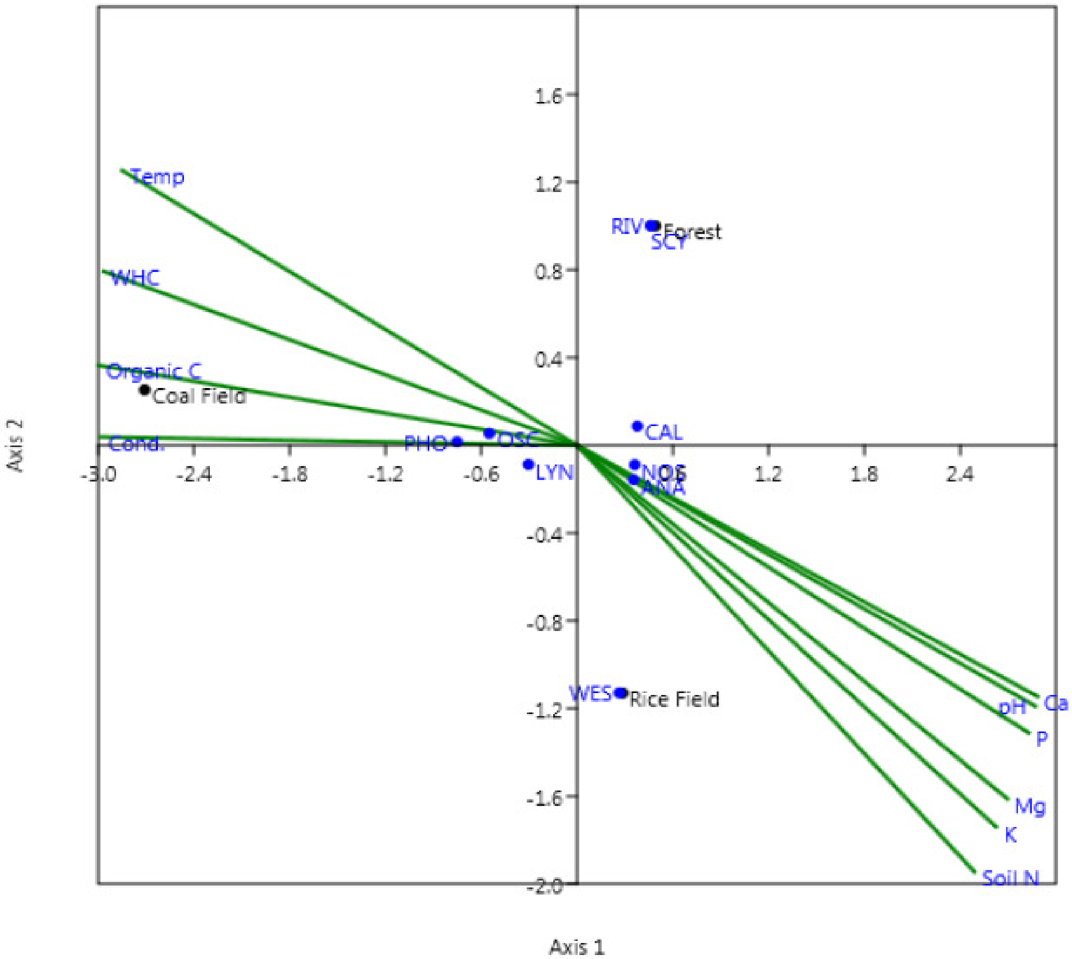

Figure 3: Canonical correspondence analysis ordination showing the relationships between the soil physico-chemical parameters and N_2-fixing cyanobacterial abundance in three land use systems - Rice fields, Reserve forests and Coalfields. Temp: Temperature, pH, Conductivity, WHC: water holding capacity, P: soil phosphate, Organic C: organic carbon, Ca: calcium, Soil N: soil nitrogen, K: potassium, Mg: magnesium; ANA- *Anabaena*, NOS- *Nostoc*, LYN- *Lyngbya*, PHO- *Phormidium*, OSC- *Oscillatoria*, SCY- *Scytonema*, CAL- *Calothrix*, RIV- *Rivularia* and WES- *Westiellopsis*.

Conclusion

The study of diversity and distribution of N_2-fixing cyanobacteria in different soil types and land use systems has relevance in the context of the soil health and fertility. The present study revealed that the soil physico-chemical parameters of different sites influenced the abundance and distribution of N_2-fixing cyanobacteria in the studied land use systems. The rice fields and reserve forests witnessed higher diversity as compared to the coalfields as the pH and nutrient availability in the soil of coalfields was comparably lower than those of rice fields and reserve forests. The

N_2-fixing cyanobacterial strains isolated from the coal field soils could further be studied to improve the quality and fertility of the acidic soils of the region.

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